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Hypothesis

Photosystem I reaction-centre proteins contain leucine zipper motifs

A proposed role in dimer formation

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The photosystem I (PS I) reaction-centre polypeptides, encoded by the *psa*A and *psa*B genes, are shown to contain several highly conserved leucine repeats, consisting of a leucine residue every seventh amino acid, similar to the leucine zipper motifs known to mediate DNA-binding polypeptide dimerisation. In each of the PSI reaction-centre subunits the leucine zipper motif precedes highly conserved cysteine residues which have been proposed to ligate the interpolypeptide [4Fe-4S] centre, Fx. We propose that PS I reaction-centre dimerisation and [4Fe-4S] centre formation are mediated through the leucine zipper.

Photosystem I; Reaction center; Leucine zipper; Iron-sulphur center

1. INTRODUCTION

Photosystem I is an integral membrane protein complex that utilizes light energy to mediate electron transfer from plastocyanin to ferredoxin. The photosystem I complex consists of at least 13 individual polypeptides, some of which bind the light harvesting chlorophyll molecules, the primary electron donor P700 and the five electron acceptors A_0, A_1, F_X, F_A and F_B [1]. The electron acceptors A_0 and A_1 have been proposed to be monomeric chlorophyll [2] and a phylloquinone [3], respectively. F_X, F_A and F_B are all [4Fe-4S] centres [1]. Both the F_A and F_B [4Fe-4S] centres have been shown to be coordinated by a 9 kDa hydrophilic polypeptide [4], and the sequence of the gene, *psa*C, has shown that the polypeptide contains 8 cysteine residues required for coordinating the two [4Fe-4S] clusters. These cysteines have a characteristic distribution typical of bacterial ferredoxins [5].

P700, A_0, A_1 and F_X are bound to a heterodimer, the photosystem I reaction-centre complex [1], consisting of the chloroplast encoded *psa*A and *psa*B gene products. *psa*A and *psa*B encode two related polypeptides with predicted molecular masses of, approximately, 83 kDa. Four cysteine residues are required for coordination of the [4Fe-4S] centre, and these may be provided by either the *psa*A product alone, or shared between the *psa*A and *psa*B products. However, since the liverwort *psa*A sequence contains only three cysteines [6], it is now accepted that coordination of the [4Fe-4S] centre, F_X, is shared between the conserved cysteines in both the *psa*A (residues 574 and 583) and *psa*B products (residues 560 and 569). Such an interpolypeptide [4Fe-4S] centre is very unusual, and whilst not without precedent (the nitrogenase polypeptide from *Azotobacter vinelandii* may also contain an interpolypeptide iron sulphur centre [7]), the model does impose some specific constraints on the interactions of the subunits in the photosystem I heterodimer.

Unlike photosystem II, for which information on the interactions of the reaction-centre heterodimer, consisting of the D1 and D2 polypeptides, can be modeled from analogies to the purple-bacteria reaction centres [8], little is known about the structure of the photosystem I reaction-centre heterodimer. In this paper we report the presence of a leucine zipper motif present in both the *psa*A and *psa*B gene products and speculate on its role in mediating interactions of the photosystem I heterodimer.

2. PHOTOSYSTEM I REACTION CENTRE POLYPEPTIDES CONTAIN LEUCINE ZIPPER MOTIFS

Leucine zipper motifs were first identified in DNA-

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A

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fos     L Q A E T D Q L E D K K S A L Q T E I A N L L
jun     L E E K V K T L K A Q N S E L A S T A N M L R
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B

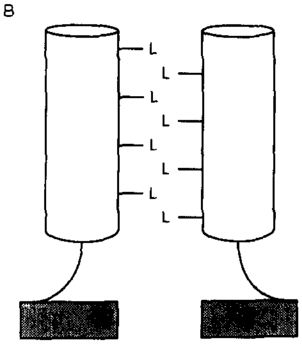

Fig. 1. (A) Amino acid sequences of the leucine repeat regions of the fos and jun oncoproteins [9]. Leucines involved in the leucine zipper are in bold. (B) Schematic of the leucine zipper showing the interaction of the leucine residues extending from the α-helix. The shaded box represents the DNA binding regions which are brought into close proximity by the leucine zipper mediated dimerisation (modified from [16]).

binding proteins such as the oncoproteins fos and jun [9] and predicted to be involved in mediating the dimerisation of these polypeptides. The leucine zipper motif is a sequence of leucine residues spaced every seventh amino acid residue along an α-helix, shown in Fig. 1A for the fos and jun polypeptides. The leucine residues are, therefore, evenly spaced every second turn of the α-helix [9]. This places all the leucine residues along one side of the helix, Fig. 1B. In the leucine zipper model, the leucine side chains extending from the zipper motif are able to interdigitate with leucine side chains of a second polypeptide which also contains the leucine zipper motif (Fig. 1B). The hydrophobic packaging of the leucine residues facilitates polypeptide dimerisation. The leucine zipper model for polypeptide dimerisation is now supported by experimental results which show that replacement of leucine amino acids, using techniques of site-directed mutagenesis, destabilises complex formation [10,11].

With the requirements for specific interactions between the photosystem I reaction-centre heterodimer polypeptides in mind, we have examined the amino acid sequences of both the *psa*A and *psa*B gene products. Surprisingly, both polypeptides were found to contain one specific region where leucine residues were equally spaced every seventh amino acid residue (Fig. 2). At least 4 leucines are present in each repeat, and this is the same number of leucine repeats found in the DNA-binding polypeptides. The leucine repeats are located in a region in which there is considerable homology between both the *psa*A and *psa*B gene products bordering the iron sulphur binding region. These leucine residues are conserved in every organism from which the *psa*A and *psa*B genes have been sequenced, including blue-green algae and liverwort, Fig. 2.

3. HYPOTHESIS

Helical wheel projections of the leucine containing regions encoded by both the *psa*A and *psa*B polypeptides, Fig. 3A, clearly shows how the leucine residues are all projected to one side of the α-helix. We propose that these conserved leucine residues are able to interdigitate in such a way as to form a leucine zipper, as

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Synechococcus  M V H H I H A F T I H V T V L I L L K G L L Y S R S S R L V P D K G Q L G F R F P C D G P G R G G T C
Liverwort      L                           L           V L F A         L I       A N L           C D G P G R G G T C
Chlamydomonas  M                           L           V L F A         L I       A N L           C D G P G R G G T C
Tobacco        L                           L           V L F A         L T       A K L           C D G P G R G G T C
Spinach        L                           L           V L F A         L I       A N L           C D G P G R G G T C
Pea            L                           L           V L F A         L I       A W L           C D G P G R G G T C
Maize          L                           L           V L F A         L I       A N L           C D G P G R G G T C

Synechococcus  L V H H A I A L G L H T T T L I L V K G A L D A R G S K L M P D K K D F G Y S F P C D G P G R G G T C
Liverwort      L             L             L             L             L           E             C D G P G R G G T C
Chlamydomonas  L             L             L             L             L                         C D G P G R G G T C
Tobacco        L             L             L             L             L                         C D G P G R G G T C
Spinach        L             L             L             L             L                         C D G P G R G G T C
Pea            L             L             L             L             L                         C D G P G R G G T C
Maize          L             L             L             L             L                         C D G P G R G G T C
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Fig. 2. Comparison of the leucine repeat containing regions in the photosystem I reaction-centre polypeptides deduced from the *psa*A and *psa*B gene sequences from *Synechococcus* [17], *Marchantia* [6], *Chlamydomonas* [18], tobacco [19], spinach [20], pea [21] and maize [22]. The conserved leucine repeats and iron-sulphur binding regions are in bold.

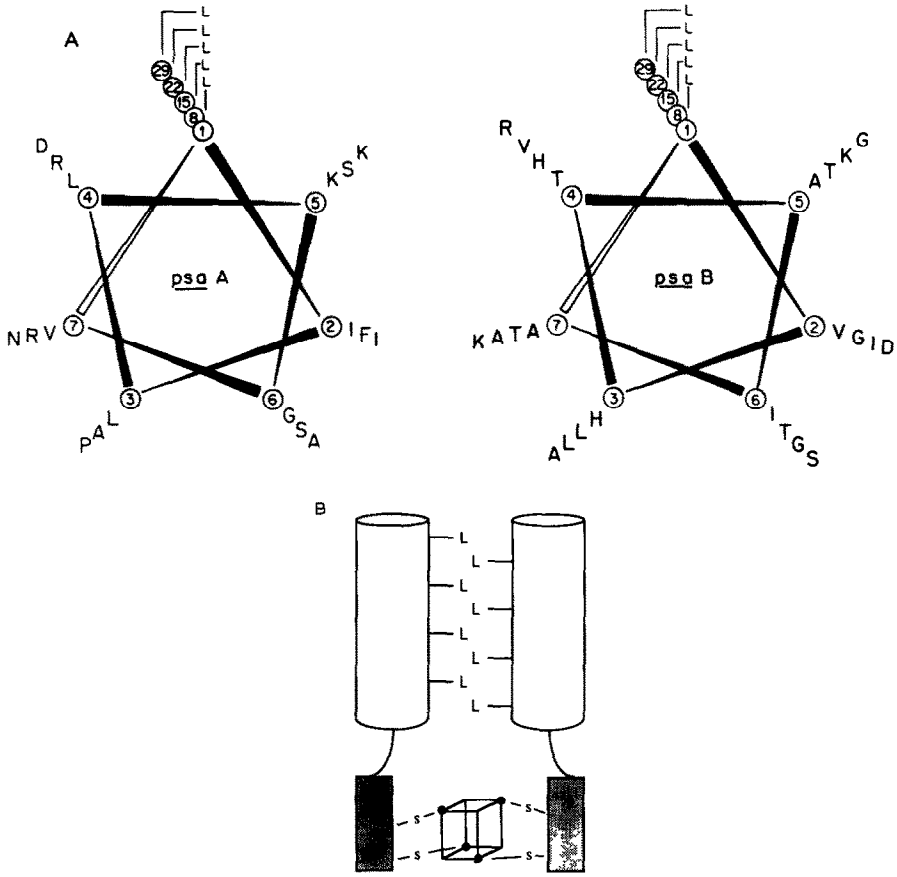

Fig. 3. (A) Helical wheel projections of the leucine zipper motif containing regions of the photosystem I reaction-centre polypeptides shown in Fig. 2. The helical wheel is drawn so that 7 amino acid residues fit into two turns of the α-helix [9]. (B) Schematic of the leucine zipper proposed for the interaction of the photosystem I reaction centre heterodimer. The model is based on the interaction of the DNA-binding polypeptides shown in Fig. 1 except that, for photosystem I, the DNA-binding regions that are brought into close proximity by the leucine zipper, are replaced by the iron-sulphur binding regions.

shown in Fig. 3B. Our hypothesis is that leucine zipper formation mediates the dimerisation of the photosystem I reaction-centre polypeptides, the *psa*A and *psa*B gene products. A leucine zipper model for the interaction of the photosystem I reaction-centre is particularly attractive because the proposed zipper region immediately precedes the iron sulphur binding region. This is very similar to the DNA binding polypeptides, where the leucine zipper precedes the regions that interact with the DNA sequence [9]. Therefore, in the same manner that the leucine zipper brings the DNA binding regions into the required proximity for activity, the leucine zipper would help to orient regions of the *psa*A and *psa*B polypeptides so that the iron-sulphur binding cysteines are in the required proximity for interpolypeptide [4Fe-4S] centre formation. As well as being a rare interpolypeptide [4Fe-4S] cluster, F_X also has an unusually electronegative redox potential [11]. This may be due to some distortion in the geometry of the [4Fe-4S] cluster [1]. The low redox potential is essential for the functioning of F_X in photosystem I, and so the proposed dimerisation between the *psa*A and *psa*B products near the [4Fe-4S] centre may also serve to provide the required geometry which allows F_X to have an extremely low redox potential.

In addition to DNA-binding polypeptides, leucine zippers have recently been reported in paramyxovirus glycoproteins [12], glucose-transporter glycoproteins [13] and voltage-gated calcium channels [14]. Leucine zippers may, therefore, be a common method of mediating polypeptide dimerisation in a wide range of different systems when very specific interactions are required. This is apparently supported by our observations of a leucine zipper motif in photosynthetic reaction centre polypeptides.

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